A NEW SPECIES OF *DIPLOCHEILA* BRULLÉ FROM NORTH AMERICA, WITH NOTES ON FEMALE REPRODUCTIVE TRACT CHARACTERS IN SELECTED LICININI AND IMPLICATIONS FOR EVOLUTION OF THE SUBGENUS *ISOREMBUS* JEANNEL (COLEOPTERA: CARABIDAE: LICININI)

KIPLING W. WILL

Department of Entomology, Cornell University, Ithaca, NY 14853, U.S.A. (e-mail: kww4@cornell.edu).

Abstract.—Diplocheila (Isorembus) crossi, new species, is described, from central and southeastern United States. Genitalic and internal female reproductive tract structures for Licinini are presented for exemplars of Dicaelina, Licinina and, Lestignathina. An evagination of the bursa common to Diplocheila and Dicaelus is a synapomorphy for Dicaelina. The spermatheca and appended gland in the subgenus Isorembus provide characters that are congruent with Ball's 1959 phylogeny for the group and suggest resolution for several ambiguous relationships. Apparent transformations of the female reproductive tract morphology include a shift from an elongate spermathecal gland and a spermatheca with a strongly differenciated tip to a spherical spermathecal gland and more uniform spermatheca. Other aspects of the tract appear to be highly conserved among the licinine taxa examined.

Key Words: character evolution, morphology, systematics

The tribe Licinini and in particular, species of *Diplocheila* have been thoroughly treated (Ball 1959, 1966, 1992). The clear diagnoses presented in those works allows for the recognition of new taxa when they are encountered. I recognize an additional specific member of the North American fauna using the criteria as defined by Nixon and Wheeler (1990), "... diagnosable by a unique combination of character states in comparable individuals".

In addition to the description of the new *Diplocheila* I present character data from the female reproductive tract. This character system has not been explored previously for the tribe Licinini although its utility for inferring phylogenetic relationships in carabids has been demonstrated in previous studies (Liebherr 1988, 1991; Liebherr and Will, in press). A cladistic parsimony analysis

combining female reproductive tract characters and characters presented by Ball (1959) was not done because the female tract was examined only for exemplars from the major clades. A complete reanalysis of Isorembus would require the development of a cladistic character matrix including many character systems and more taxa. This is beyound the scope of this study. In order to investigate character transformation the female reproductive tract characters are mapped onto the existing phylogenentic hypothesis. Characters were found largely congruent with Ball's hypotheses for Licinini (Ball 1959, 1966, 1992). Characters supporting relationships within the striatopunctata group were ambiguous in Ball's (1959) work and so female tract characters used here provide synapomorphies for sistergroup relationships among those taxa.

METHODS

External structures of the type series of Diplocheila crossi were examined using a dissecting stereo-microscope at magnification of 100× or less. Male genitalia were removed after softening the specimens in near boiling soapy water and then gently cleared in cold 10% KOH. Females were softened in near boiling soapy water, whole abdomens removed and placed in a ceramic spot plate in 10% KOH at either room temperature for 24 hours or in warm 10% KOH for 5–10 minutes. Further dissection was then done in a 10% acetic acid solution. The neutralized abdominal apex plus reproductive tract was then place in a saturated Chlorazol Black® dye and methyl cellosolve solution. Stained tracts were examined with the dissecting microscope under glycerine in a ceramic spot plate to determine the three dimensional aspects of structures. Tracts were then slide mounted in glycerine and examined using a phase-contrast compound microscope. Drawings were made using an ocular grid. Nomenclature of female reproductive tract structures follows Liebherr and Will (in press).

Diplocheila (Isorembus) crossi Will, new species (Figs. 1, 2A–2C, 3, 4, 10)

Diagnosis.—In contrast to other species of North American *Diplocheila*, this species and *D. striatopunctata* LeConte share a prominent tubercle on the dorsal surface of the left mandible. The distinct shape of the relatively larger pronotum (Fig. 1), widest behind the middle with the sides smoothly arcuate almost to the base, together with the form of the aedeagus, which is blunter at the tip and lacks any sign of a depression vertrally, (Fig. 2 a–d) separates *D. crossi* from *D. striatopunctata*.

Description.—Overall length 12.5–14.5 mm (anterior tip of labrum to apex of ely-tra).

Head (Fig. 1): Broad, rather quadrate, ocular ratio (greatest width over eyes/great-

est width between eyes) 1.47-1.50, piceous, shiny, microsculpture very faint isodiametric with scattered punctulae visible at $>60\times$, glabrous except for normal pair of supra-orbital setae over each eye, 1 seta at anterolateral corner of clypeus and 4 setae on labrum, Clypeus, margin of labrum, maxillary and labial palps and antennae paler, rufopiceous, sometimes slightly infuscated but never as dark as vertex of head; labrum deeply and asymmetrically emarginate, right side more prominently produced; mandibles broad, asymmetrical, right smooth, left with prominent dorsal protuberance; labial and maxillary palpomeres fusiform, glabrous except for stout pair of medial setae and a minute terminal seta on penultimate labial palpomere, Antennae reaching well past base of pronotum, basal antennomeres with 1 long subterminal seta, third with a ring 4-5 smaller setae, segments IV-XI densely pubescent except for thin, interior and exterior glabrous stripes, segments elongate, length $3.7-3.9 \times$ width.

Thorax (Fig. 1): Pronotum distinctly wider than long, median length/width at widest point 1.34-1.50, widest just behind midpoint; piceous, all margins thinly paler rufo-piceous, shiny, slightly duller than head due to more evident microsculpturing and punctulae, lateral margins smoothly curved to hind angles, hind angles slightly more obtuse than right, weakly or not produced, lateral bead slightly widening to apical margin, base unmargined and produced at inner basal impressions; inner basal impressions distinct and linear, outer impressions broadly and weakly impressed, lateral setae at apical third, basal setae set close to corner of hind angle, Proepisternum dull from strong isodiametric sculpticells, Prosternal process with a slight medial depression, tip of process strongly margined, Elvtra broad, 1/w 1.47-1.50, depressed, piceous, feebly shiny, microsculpture as in pronotum, epipleura with apical-two thirds paler, rufopiceous; humeri with minute tooth, shoulders prominent, stria 1-6 im-



Figs. 1–3. 1, 2A–C, 3, *Diplocheila crossi*, 2D, *D. striatopunctata*. 1, Head and pronotum. 2A, Median lobe, left lateral. 2B, Median lobe, ventral. 2C, Median lobe, dorsal. 2D, Tip of median lobe, left lateral. 3, Metathoracic wing.

pressed, though often weaker apically and/ or not reaching elytral base, striae smooth or with weak, elongate punctures, stria 7 faintly impressed, a single dorsal puncture on each elytron just behind midline on third interval or touching second stria; Flight wing (Fig. 3) large, length from axil to tip/ width at widest point 2.57 (n = 1), fully reflexed at tip, Ventral pterothorax with strong microsculpture, isodiametric laterally, stretched mesh medially; metepisternum elongate, anterior width/length ratio 1.60, Hind tibia with 3–4 spines in posterior median row, Tarsomere V glabrous ventrally.

Abdomen: Ventrites with strong isodiametric microsculpture, male with 2, and female with 4 setae at apical margin of last visible ventrite, Male genitalia (Fig. 2 a–c):



Figs. 4–7. Female reproductive tract, ventral view. 4. *Diplocheila crossi.* 5, *D. polita.* 6, *D. zeelandica.* 7, *Dicaelus teter.* (bc = bursa copulatrix; co = common oviduct; al = apical lobe; gc1 = gonocoxite 1; gc2 = gonocoxite 2; sg = spermathecal gland; sp = spermatheca; vc = villous canal)

Aedeagus with tip rounded, shaft with apical half ventrally and laterally smooth, basal half weakly strigous, smoothly convex medio-ventrally, ostium dorsal, Female genitalic and reproductive tract (Fig. 4): Laterotergite IX triangular with prominent apical setae. Gonocoxite-I with 3 medial and 2 ventral setae. Gonocoxite-II without ensiform setae, and with 2 nematiform setae. Bursa copulatrix forming a cup with junction of spermatheca and common oviduct telescoped within. Bursa with distinct



Figs. 8-9. Female reproductive tract, ventral view. 8, Badister reflexus. 9, Siagonyx sp.

ventral lobe at base of oviduct. Spermatheca with broad base and long ribbon-like apical horn. Base and apex of similarly thin cuticle. Villous canal well developed, running from near base of spermatheca and terminating well up common oviduct. Spemathecal gland duct inserts basally into spermatheca; Gland has a distinct ampulla and spherical reservoir.

Types.—Holotype: 33°10'07"N, 90°18' 36"W, USA, MISSISSIPPI, Washington Co., Leroy Percy S.P., 23-V-1995, Cols. K. Will & R. Androw. (9, CUIC) Allotype: Florida, Jackson Co., Spring Lake, 10 Jul[y] 1981, W. H. Cross, Collected at artificial light (d, MUIC) Paratypes: Same data as holotype (\mathcal{Q} , KWW collection); same locality data as allotype plus second label with "blacklight trap in deciduous woods" (9, MUIC); ILLINOIS, Henry Ulke Coll. (2 ♂, 2 ♀, CMNH); Johnson Co., Heron Pond, May 1 1976, L. R. Davis Jr. (2 9, 1 8, CMNH); Alexander Co., Horseshoe Lake, 30 April 1976, L. R. Davis Jr. (d, CMNH); MISSISSIPPI, Washington Co. Leroy Percy St. Pk., 5 mi. W. Hollendale, D., M. & E. Hildebrandt, T15N, R7W, Sec. 5, under logs, boards in hardwood swamp (12 April 1997, 2 δ , 5 \Im ; 14 April 1997, 3 \Im , D. Hildebrandt Collection), TENNESSEE, Shelby Co., Meeman Biolog. Sta., 20 km N Memphis, July 1–10 1991, R. Ritke (\Im , CMNH).

Etymology.—The species is named in honor of William H. Cross, an excellent general collector, from Mississippi State University. R. A. Androw and I were on our way to participate in a collecting expedition in his name when the holotype and one paratype were collected. Additionally, the allotype and one paratype were collected in 1981 by Dr. Cross.

Affinities.—*Diplocheila crossi* shares characteristics of the head, concave front and clypeus and asymmetrically emarginate labrum, with other members of *Isorembus* and it shares the following apomorphies with the *striatopunctata* group as defined by Ball (1959): gonocoxite-II without ensiform setae, prosternal apex margined, and tarsomere V without ventral setae. The appended gland is spherical as in *D. major major* and *D. striatopunctata*. The dorsal tubercle of the left mandible and the strigous medial lobe are synapomorphies for



Fig. 10. Distributional records for Diplocheila crossi.

D. crossi and *D. striatopunctata* (Fig. 11, characters 5–6).

Geographic range.—The 23 specimens in the type series suggest a range (Fig. 10) that is restricted to the Gulf coast and lowlands of the Mississippi drainage.

Ecology.—The holotype and one paratype were collected in a swampy area with highly organic soil. Several species of *Brachinus, Chlaenius, Loxandrus* and, *Lophoglossus* were also collected in this same habitat.

ANALYSIS AND DISCUSSION OF CHARACTERS OF THE FEMALE REPRODUCTIVE TRACT

Taxa and methods.—Females were examined for the following species; *Badister reflexus* LeConte, *Licinus punctatulus* F., *Dichrochile goryi* Boisduval, *Siagonyx* sp., *Dicaelus polita* Dejean, *D. teter* Bonelli, *Diplocheila* (s.str.) *polita* F., *D. (Isorembus) zeelandica* Redtenbacher, *D. (I.) cordicollis* Laferté, *D. (I.) aegyptiaca* Dejean, *D. (I.) obtusa* LeConte, *D. (I.) striatopunctata* LeConte, D. (I.) crossi new species, D. (I.) nupera Casey, and D. (I.) major LeConte.

The licinine female reproductive tract morphology is conserved in all taxa examined (Figs. 4–9). The basic arrangement includes a thick walled, cup-shaped bursa. The common oviduct and spermatheca apically join the bursa and are telescoped into the concavity of the cup. The spermatheca is curled or coiled, at least at the tip. The villous canal runs between the base of the spermatheca and onto the common oviduct. This canal can be more or less developed, but is present at least as a small patch. A gland is appended to the spermatheca with its duct entering into the basal third of the spermatheca.

The reproductive tract of Dicaelina (Figs. 4–6). Ball (1992) provides synapomorphies for all the licinine subtribes except for Dicaelina. He stated that characters were ambiguous regarding resolution of the basal polytomy of *Diplocheila*, *Dicaelus* and (Licinina (Lestignathina + Dicrochilina). He

posited a working hypothesis that *Diplocheila* + *Dicaelus* form a monophyletic Dicaelina and suggested that characters systems other than those previously explored may yield synapomorphies for the clade. Among taxa examined only *Diplocheila* and *Dicaelus* were found to have a apical lobe on the bursa copulatrix (al, Figs. 4, 7). This character is considered a synapomorphy for the subtribe (Fig. 11, character 1).

The differentiated tip of the spermatheca is several times longer than the spermathecal base in Dicaelus and most Diplocheila species. However, both D. polita and D. *zeelandica* have a very short tip relative to the base (Fig. 11 character 2). This could be interprted as a synapomorphy of these taxa making Isorembus paraphyletie relative to Diplocheila s.str. However, given the suite of characters supporting the monophyly of the subgenus Isorembus (i.e., modifications of clypeus, labrum, mandibles [Ball 1959, 1992] and ovoid or spherical appended spermathecal gland) the striking similarity of spermathecae in Diplocheila (s.str.) polita and D. (I.) zeelandica can be interpreted as convergence. Shortening of the spermathecal tip in *Diplocheila* s.str + *Isorembus* and then the reaquasition of a long tip in Isorembus minus D. zeelandica is an equally possible optimization of this character transformation. Convergence to the reduced form is preferred as a working hypothesis based on the assumption that reduction to a simpler form seems more plausible than the reappearance of the elongate coil. Sampling of more taxa from Diplocheila s.str. and the zeelandica group may provide an unambiguous optimization for this character without the need for assumption of process.

The shape of the spermathecal gland varies from elongate, many times longer than wide (Fig. 7); to ovoid, 2–4 time longer than wide (Fig. 6); to spherical, of approximately equal length and width (Fig. 4). *Dicaelus* and *Diplocheila polita* have elongate glands. Members of *Isorembus* have ovoid or spherical glands (Fig. 11, character 3). The ovoid state is found in the basal *lso-rembus* species (*zeelandica* group, *aegyp-tiaca* group, *D. obtusa*, *D. mupera*). A spherical gland is a synapomorphy for *Di-plocheila major major*, *D. crossi*, and *D. striatopunctata* (Fig. 11, character 3').

In all Dicaelina the base of the spermatheca is expanded relative to its tip. A similar condition is found in Badister (Fig. 8) and Dicrochile. Long, undifferentiated and spiral spermathecae are found in Licinus and Siagonyx (Fig. 9). Possible adelphotaxa to Licinini, e.g. Panagaeini, Orthogonini, and Melanochitonini do not have spiral form spermathecae and do not show any differentiation between the base and tip of the spermatheca (Liebherr and Will, in press). The primative condition of the spermatheca in licinines is ambiguous. The base-tip differentiation could be interpreted as a synapomorphy for Dicaelina. However, since discrete states are difficult to assign. assesment of homology remains unclear at this taxonomic level.

Within Dicaelina, *Dicaelus, Diplocheila polita, D. zeelandica*, and species of the *aegyptiaca* group have the spermathecal base well developed, thick, highly sculptured (when without spermatophore) and sharply contrasting with a smooth, horn-like spiral tip. The spermatheca of the *striatopunctata* group (Fig. 11, character 4) has an expanded basal area, but this is not of a fundamentally different composition from that of the spiral horn-shaped tip.

CONCLUSIONS

The addition of new taxa and characters provides the only real test of phylogenetic hypotheses. The addition of *D. crossi* and examination of characters of the female reproductive tract increases the robustness of previous phylogenetic hypotheses for licinines. Morphological studies have been and will continue to be the basis for the vast majority of stable, natural classifications. New character systems and new methods of interpretation are the bricks and mortar of systematics. With careful dissection and in-



Fig. 11. Cladogram of selected Licinini. Characters: I = ventral lobe of bursa present: 2 = spermatheca with short tip; 3 = appended gland ovoid; 3' = appended gland spherical; 4 = spermathecal base and tip differentiated; 5 = mandible with prominent tubercle; 6 = median lobe strigous. See text for explanation of characters.

terpretation, the female reproductive tract of carabids can provide characters useful for the building or buttressing of phylogenetic hypotheses.

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